REMARKS

Applicant gratefully thanks Examiners Leavitt and Woitach for taking the time on February 23, 2010 for a very productive personal interview with Farhad Parhami and applicant's representative. Applicant points out that the Interview Summary sent on February 25, 2010 listed some possible claim amendments that were discussed, but that these potential claim amendments were not binding. Applicant is in general agreement with the substance of the Interview Summary.

The claims

Some of the claims (*e.g.*, claims 1 and 6) have been amended to clarify that, at least to the extent that the claims read on *in vivo* administration of an oxysterol, the oxysterols are selected from the group consisting of 20S-hydroxycholesterol, 22S-hydroxycholesterol, 22R-hydroxycholesterol and 25-hydroxycholesterol, or active portions thereof. Claims directed to the administration of an oxysterol to a patient (*e.g.*, claims 16, 19 and 29) are amended to clarify that the patient is suffering from bone loss and/or requires bone repair. The amendments do not add new matter, and do not narrow the scope of the claims.

Applicant requests that, once the claims currently under consideration are allowed, the claims reciting species which were not elected for preliminary examination (*e.g.*, claims 4, 5, 9 10, 18, 22 and 27) be examined. It is noted that claims 4, 5, 9 10, 18, 22 and 27 recite additional elements that were not recited in the examined claims. That is, they recite "further comprising" treating the mammalian mesenchymal cells with at least one secondary agent. Therefore, provided that the elected claims are deemed patentable, these additional claims, which merely add additional elements or steps, will not require a further search and examination, and should also be deemed to be patentable.

Introduction

At the outset, applicants wish to clarify the distinction between the process of inducing osteoblastic differentiation of mammalian mesenchymal stem cells (MSCs), which is sometimes referred to as osteoinduction or osteogenesis, and the process of osteoconduction. Osteoinduction, which is recited in the present claims, is the process by which stem cells (*e.g.*,

pluripotent or multipotent stem cells) are induced to differentiate into osteoblasts. Once stem cells have been induced to differentiate into osteoblasts, they express markers of osteoblastic differentiation. Markers of osteoblastic differentiation include, *e.g.*, an increase in alkaline phosphatase activity, the expression of osteocalcin mRNA expression, or mineralization. Thus, MSCs that have been induced to differentiate into osteoblasts become committed to the osteoblast lineage and do not undergo differentiation into a different lineage, *e.g.* adipocyte differentiation. For example, as recited in claim 1, an oxysterol of the invention both induces osteoblastic differentiation *and* inhibits adipocyte differentiation of the MSCs. In contrast, osteoconduction occurs after osteoinduction has taken place. In osteoconduction, cells already express markers of osteoblastic differentiation (*e.g.*, the immortalized osteoblast cell lines, MC2T3 [MC3T3] and MG-63) and, are, by definition, no longer mesenchymal stem cells (pluripotent or multipotent cells) that can be induced to undergo osteogenesis, but rather, osteoconductive cells are limited to osteoblastic proliferation or further maturation along the osteoblastic pathway. Recognition of the difference between these two processes is important for determining whether a reference discloses that an agent "induces osteoblastic differentiation," as is presently claimed, or whether it discloses a different process: the process of osteoconduction.

Claim Rejections Under 35 U.S.C. § 103(a)

Claims 1-3, 6-8, 11-17, 19-21, 23-26, and 28 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Paralkar *et al.* (U.S. Patent Application No. 2004/0176423; hereinafter referred to as "**Paralkar**") in view of Parish *et al.* (1995) *Lipids*, 247-251, hereinafter referred to as "**Parish**", and further in view of Wang *et al.* (2000) *Clinical Orthopedics and Related Research* 370, 295-310, hereinafter referred to as "**Wang**".

Applicants respectfully traverse this basis for rejection and submit that the Action fails to provide a sufficient basis for one having ordinary skill in the art to predictably arrive at the presently claimed invention with any reasonable expectation of success. Thus, the Action fails to establish a *prima facie* case of obviousness against the presently claimed invention.

As a preliminary matter, the Examiner repeatedly alleges in the Action that "one cannot show non-obviousness by attacking references individually where the rejections are based on a combination of references." Applicants respectfully note that a proper analysis for obviousness requires that "the scope and content of the prior art [be] ... determined; differences between the

prior art and the claims at issue [be] ... ascertained; and the level of ordinary skill in the pertinent art resolved." *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966). Thus, the analysis must include a discussion of the prior art on which the Examiner is relying. Just as the Examiner has addressed the references individually in setting out the rejection, Applicants offer in rebuttal, a discussion of what the individual references disclose. This rebuttal discussion cannot be construed as an attack on the references individually; it is simply a discussion of what the individual references disclose so as to ascertain whether the combined teachings support the Examiner's assertion of obviousness.

Applicants respectfully submit that the Action has failed to establish a *prima facie* case of obviousness with respect to the presently claimed subject matter (See *In re Mayne*, 104 F.3d 1339 (Fed. Cir. 1997); the USPTO has the burden of showing a *prima facie* case of obviousness). The Examiner must at a minimum demonstrate that the combined references teach or suggest all the claim features (*See In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)); and even assuming, *arguendo*, that the combination of references teaches each claim feature, the Examiner must provide an explicit, apparent reason to combine these features in the fashion claimed by the Applicant with a reasonable expectation of success. *See KSR v. Teleflex, Inc.*, No. 04-1350 at 4, 14 (U.S. Apr. 30, 2007) ("A patent composed of several elements is not proved obvious merely by demonstrating that each element was, independently, known in the prior art").

The Examiner's line of reasoning is insufficient to establish a *prima facie* case of obviousness, at least because the Examiner has provided no technical evidence or reasoning to support the conclusion that the skilled artisan would have a reasonable expectation of success of using oxysterols to simultaneously inhibit adipocyte differentiation and induce osteoblastic differentiation of a mesenchymal stem cell. *See KSR v. Teleflex, Inc.* at 14, citing In re Kahn, 441 F.3d 977, 988 (Fed. Cir. 2006) ("[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.").

Here, the Examiner alleges that it would have been obvious to use oxysterols to simultaneously inhibit adipocyte differentiation and induce osteoblastic differentiation of a mesenchymal stem cell because statins inhibit HMG-CoA reductase and allegedly induce osteoblastic differentiation (Paralkar); because oxysterols inhibit HMG-CoA reductase (Parish); and because statins inhibit adipocyte differentiation in mesenchymal stem cells (Wang).

Assuming, *arguendo*, that the Examiner's characterization of Paralkar, Parish, and Wang were accurate, one having skill in the art would still not have a reasonable expectation of success in arriving at the presently claimed invention because the Examiner has provided no technical evidence or reasoning to support a causal link or nexus between inhibition of HMG-CoA, by statin, an oxysterol, or any other compound, and simultaneously inhibiting adipocyte differentiation and inducing osteoblastic differentiation of a mesenchymal stem cell.

The Action alleges that cell culture of progenitor osteoblastic cells with statin stimulated bone formation *in vitro* and enhances osteoblast cell numbers at all stages of differentiation (p.3, paragraphs [0011] and [0051]). The Action also alleges that Paralkar teaches methods of stimulating mammalian cells to express biological differentiation markers (p.3, paragraph [0053]). The Action concludes that Paralkar teaches that statins inhibit HMG-CoA reductase which leads to increased osteoblastic differentiation.

Applicants respectfully submit that Paralkar attributes the foregoing observations, in part, to Harris, *et al.*, (1995) *Mol. Cell. Differ.* 3, 137, for its disclosure that BMP is an important factor for the induction of osteogenesis; **Mundy**, *et al.* (1999) *Science* 286, 1946; and U.S. Pat. No. 6,080,779 (the " **'779 patent**," from the Mundy laboratory). These references are discussed as background information in Paralkar; thus, the observations of these references are not "taught" by Paralkar. The teachings of these references are discussed individually below.

Applicant notes that the "teachings" of Paralkar, itself, are restricted to administering a combination of at least two compounds, a statin and a prostaglandin receptor agonist (see, *e.g.*, paragraphs 22-28, 61-70, 76, and claim 1). In fact, nowhere in the entirety of Paralkar does this reference teach or suggest that the administration of a statin alone would be useful for enhancing bone formation in a mammal.

The primary references discussed in **Paralkar** are the Mundy *et al.* paper (hereinafter referred to as "**Mundy**") and the related **'779 patent**. The following discussion relates specifically to the Mundy paper, but also applies to the '779 patent. Mundy discloses that contacting immortalized osteoblast cell lines (murine 2T3 cells (Ghosh-Choudhury *et al.*, 1996. *Endocrinology*, Vol 137, 331-339); murine MG-63 osteoblasts (see Figure 1 of Mundy)) with statins results in increase BMP-2 expression. Importantly, as discussed in the Introduction section above, the immortalized osteoblast cell lines of Mundy are not mammalian mesenchymal

stem cells (MSCs). Not only does Mundy fail to disclose experiments using MSCs, but a skilled artisan would not have had a reasonable expectation of success in simultaneously inhibiting adipocyte differentiation and inducing osteoblastic differentiation, based on conclusions derived from experiments using immortalized osteoblast cell lines, because such cell lines are not multipotent and, instead, are committed to the osteoblastic lineage.

In addition to studies with immortalized osteoblast cell lines, Mundy discloses that explanted caveolar bone from mice treated with statins, BMP-2, or FGF increase the number of bone cells in tissue explants. However, Mundy does not demonstrate the induction of osteogenesis in these calvarial samples. The legend of Figure 2 in Mundy states that "There is marked cellular proliferation and accumulation of mature osteoblasts adjacent to new bone in the bones treated with simvastatin." (emphasis added). The histological criteria used by Mundy could not have identified the induction of osteogenesis. One having skill in the art could only reasonably conclude from Mundy *et al.* that statins, BMP-2, and FGF have a role in osteoconduction, which takes place after the induction of osteoblastic differentiation (i.e., osteoinduction). The osteoconduction could include osteoblast proliferation, which would account for the increased number of osteoblasts, and/or the maturation of osteoblasts. Applicants submit that these effects are inconsistent with the presently claimed function of an oxysterol; an oxysterol that simultaneously inhibits adipogenic differentiation and induces osteoblastic differentiation in a mesenchymal stem cell does not increase the number of differentiated cells but rather differentiates a multipotent cell into an osteoblastic cell.

For at least the preceding reasons, it is clear that Mundy does not disclose the induction of osteogenesis and the inhibition of adipogenesis of MSCs, either in cell culture or in calvarial explants.

The Examiner further cites Mundy for its alleged report that statins both inhibit HMG-CoA reductase activity and induce osteoblastic differentiation (the latter of which is actually not disclosed by Mundy, as is discussed above); and the Examiner alleges that, because oxysterols have also been reported to inhibit HMG-CoA reductase activity, it would be obvious to a skilled worker from Mundy that oxysterols would also be expected to induce osteoblastic differentiation. However, there is a logical disconnect to the allegation that oxysterols could substitute for the statins of Mundy, because Mundy did not demonstrate a causal relationship between the ability of statins to inhibit HMG-CoA reductase activity and their alleged ability to

induce osteoblastic differentiation. Thus, even if it were the case that certain statins, which can inhibit HMG-CoA reductase, could induce osteoblastic differentiation in MSCs, this does not necessarily mean that OTHER agents which inhibit HMG-CoA reductase, such as oxysterols, would also induce osteoblastic differentiation in MSCs.

Moreover, Paralkar is silent with regard to the use of an oxysterol, a statin, an HMG-CoA inhibitor, or any other compound, to simultaneously inhibit adipogenic differentiation and induce osteoblastic differentiation in an MSC as presently claimed.

Biological phenomena, such as the induction of osteoblastic differentiation and the inhibition of adipocyte differentiation, are complex and not completely understood. It would have been unpredictable at the time of filing the present application whether two different inhibitors of HMG-CoA reductase would have the same effect on, *e.g.*, osteoblastic differentiation. HMG-CoA reductase could be eliciting any of a variety of effects on a cell, which might or might not be associated with bone formation or osteogenic differentiation. Absent the establishment of a causal link between the ability of an agent to inhibit HMG-CoA reductase and the ability of the agent to induce osteoblastic differentiation, there would have been no motivation for a skilled worker to substitute an oxysterol for the statins of the Mundy reference, with the requisite reasonable expectation of success.

Importantly, Rao *et al.* (1999) *Proc Natl Acad Sci USA* 96, 7797 (hereinafter referred to as "**Rao**"), a copy of which is filed in an IDS herewith, reports that one of the statins used by Mundy, lovastatin, although it had been shown to inhibit HMG-CoA reductase activity, exerts a biological effect (the inhibition of cell division by G1 arrest) via a mechanism that is entirely unrelated to the cholesterol biosynthetic pathway. This report, in itself, refutes the notion that the ability of a statin to inhibit HMG-CoA reductase activity is necessarily causally linked to a particular biological effect in a cell.

In brief: as support for an alleged causal connection between the inhibition of HMG-CoA reductase activity and the stimulation of bone growth, Mundy reports (page 1946, right column) that the administration of the statin, lovastatin, leads to increased expression of a reporter gene linked a BMP-2 promoter (which, according to Mundy, is the basis of, and a marker for, the stimulation of bone growth in its system); but that this increase in a marker for bone growth can be blocked by the addition of the immediate downstream metabolite of HMG-CoA reductase, mevalonate. Mundy hypothesizes (page 1946, last paragraph) that this observation "suggests

that effects on bone formation were causally linked to inhibition of this enzyme." However, **Rao** (which is referred to in the Mundy paper) discloses another biological phenomenon, in which an effect mediated by the statin, lovastatin, - in this case, the inhibition of cell division by G1 arrest - can also be abrogated by mevalonate. In this case, however, Rao reports that the mevalonate actually acts, not via the cholesterol biosynthetic pathway, but by inhibiting a completely unrelated activity, proteasome activity. Rao states on page 7802, last paragraph, that "We have provided evidence that lovastatin suppresses cell proliferation through inhibition of proteasome-mediated degradation of p21 and p27, and mevalonate can abrogate this effect by activation of the proteasome."

Thus, not only does Mundy fail to show that a statin can induce osteoblastic differentiation (and consequently inhibit adipogenesis) of MSCs; but also, particularly in view of Rao, Mundy fails to establish a causal connection between the ability of a statin (or other agent) to inhibit HMG-CoA reductase activity and the stimulation of bone formation. Mundy certainly does not establish a causal connection between the ability of a statin (or other agent) to inhibit HMG-CoA reductase activity and the stimulation of *osteogenesis* (with the concomitant inhibition of adipogenesis).

The "**Parish**" reference does not remedy the defects of Paralkar (in particular, the Mundy references).

The Action acknowledges that Paralkar does not specifically teach osteoblastic differentiation with an *oxysterol*. However, the Examiner refers to Parish for its disclosure that side-chain oxysterols are potent inhibitors of HMG-CoA reductase. Applicant submits that Parish fails to mention osteoblastic differentiation and is completely silent with regard to any nexus between HMG-CoA reductase inhibition and osteoinduction. Thus, even if Parish discloses that side-chain oxysterols are potent inhibitors of HMG-CoA reductase, it does not remedy the defects of Paralkar, *e.g.*, the failure to show the induction by an oxysterol of osteoblastic differentiation in MSCs and the failure to show that an oxysterol (or, for that matter, any particular inhibitor of HMG-CoA reductase) would be expected to induce osteoblastic differentiation. Furthermore, Parish does not suggest or disclose that an inhibitor of HMG-CoA

reductase can inhibit adipocyte differentiation of mammalian mesenchymal stem cells (as required by claim 1).

The **"Wang"** reference does not remedy the defects of Paralkar, or of Paralkar taken with Parish.

The Examiner cites Wang for its alleged disclosure that treatment with statins (in this case, lovastatin) inhibits adipocyte differentiation and induces osteoblastic differentiation of mouse MSCs, as indicated by markers such as alkaline phosphatase activity, osteocalcin mRNA and cAMP production. In particular, Wang is cited for its disclosure of differentiation of MSCs.

For at least the reasons presented in the Reply filed on September 23, 2009, which is incorporated by reference in the present Reply, Wang does not remedy the defects of Paralkar, or of Paralkar taken with Parish, *e.g.* the failure to teach that an oxysterol (or, for that matter, any particular HMG-CoA reductase inhibitor) would be expected to induce osteoblastic differentiation in MSCs. Like the other references cited by the Examiner, Wang is directed to the effects of a statin, and does not suggest or disclose that an oxysterol could substitute for the statin to, *e.g.*, induce osteoblastic differentiation.

Furthermore, applicant notes that the D1 cells used in Wang are *not* MSCs, in spite of the indication by Wang that these cells are allegedly "pluripotent." This is clearly shown, for example, in Figure 2. The control cells, which were not treated with a statin, are *already* producing significant amounts of markers of cell markers of differentiated osteoblasts: collagen and osteocalcin. Thus, these D1 cells have clearly already been induced to differentiate into osteoblasts and are thus committed to the osteoblastic lineage, even before having been subjected to treatment with a statin.

Moreover, Wang fails to demonstrate that a statin (*e.g.*, lovastatin), by itself - and not co-administered with dexamethasone - can affect either adipogenesis or osteoblastic differentiation. On page 297 (column 1, last paragraph through column 2, first paragraph), page 299 (column 1), p. 300 (column 1), and p. 307 (column 2, first full paragraph), cited by the Examiner, Wang indicates that lovastatin was administered in conjunction with the steroid dexamethasone to determine whether lovastatin reduced the stimulation of an adipogenic marker and the inhibition of osteogenic markers by dexamethasone. Wang did not consider the effect of lovastatin alone on the cells. For example, Figure 2 of Wang shows the effect of a control, dexamethasone alone,

and a combination of dexamethasone and lovastatin on markers of adipogenesis and osteogenesis, but does not present the effect of lovastatin alone on cells. One of skill in the art could understand Wang's observed effect of lovastatin in reducing the stimulation of an adipogenic marker and inhibition of osteogenic markers by dexamethasone as being consistent with lovastatin interfering with the effect of dexamethasone, rather than lovastatin independently stimulating osteogenesis.

In fact, Wang state and provide data to support the conclusion that chickens treated with lovastatin *alone* did not have any measurable change in fat area or bone mass when compared to control treated chickens (see, *e.g.*, Table 2, p. 306; compare groups C and D). At least because Wang uses cells that are already committed to the osteoblastic lineage, and does not show that treatment with a statin alone induces osteoblastic differentiation and inhibits adipocyte differentiation of mesenchymal stem cells, Wang does not remedy the deficiencies of Paralkar (*e.g.*, the two Mundy references) and Parrish, taken separately or together, which, *e.g.*, do not teach oxysterol administration to mesenchymal stem cells. Accordingly, Applicant submits that the instant claims 1 and 6 are nonobvious over Paralkar (particularly the Mundy references), Parrish and Wang.

The failure of Wang and colleagues to demonstrate that statins can induce bone formation is supported by the characterization of Wang's studies by Mundy (in Mundy's '779 patent). Column 2, last paragraph of the '779 patent indicates that Wang *et al.*, *J. Formos Med Assoc* (1995) 94:589-592 report that there was no effect on bone formation in a rabbit model when the rabbits were administered lovastatin in the absence of steroid treatment. The inventors of the `779 patent conclude (column 3, first paragraph) that "there is no suggestion in Wang that lovastatin directly enhances bone formation."

In addition, the `779 patent states that Cui *et al.*, *J Bone Mineral Res.* (1996)11(S1):S510 (also from the Mundy laboratory) report that chickens that received a combined treatment of dexamethasone and lovastatin had improved bone growth, measured in the femoral head, compared to chickens that had undergone bone loss as a result of dexamethasone treatment alone. The inventors of the '779 patent conclude (col. 3, second paragraph) that "there is no suggestion that lovastatin directly enhances bone formation in the absence of steroid treatment." In fact, no increase in bone mass was seen chickens administered lovastatin alone compared to

control chickens (see Cui *et al.*, *Clin Orthop Relat Res.* 1997 Nov;(344):8-19; data presented and discussed in Wang, on page 305).

Furthermore, the literature contains counterexamples of statins that do not promote or inhibit bone growth. Wada *et al.* (2000) *Arch. Intern. Med.* 160, 2865 (copy filed herewith in an IDS) reports that the mean bone mineral density was lower in human subjects receiving pravastatin, simvastatin, or fluvastatin than in those not receiving statins. Also, as noted in the Reply filed on September 23, 2009, Parhami *et al.* (2002) *J. Bone Mineral Res.* 17, 1997-2003, report that lovastatin and mevastatin suppressed the expression and activity of alkaline phosphatase and inhibited calcium mineral deposition in pluripotent marrow stromal cells. That is, lovastatin and mevastatin inhibited markers associated with osteogenesis. Therefore, one of ordinary skill in the art would not have had a reasonable expectation that inhibition of HMG-CoA reductase (either by a statin or another compound) would induce osteoblastic differentiation.

In addition, none of the cited references, taken alone or in combination with other cited references, suggests or discloses that the particular oxysterols or combinations of oxysterols that are recited in the present claims (*e.g.*, claims 1, 3, 6, 8, 16-17, 20-21, or 25-26) can induce osteoblastic differentiation and inhibit adipocyte differentiation of MSCs.

For at least the preceding reasons, neither the cited references nor the art at the time the invention was made provide a motivation to combine the cited references to achieve the presently claimed invention, with a reasonable expectation of success, and thus do not render the present claims obvious. Applicant requests that the obviousness rejection be withdrawn.

Obviousness-type double patenting rejections

Applicant, without acquiescing to any rejection, respectfully requests that the two provisional double-patenting rejections be held in abeyance until the allowance of claims in the instant application. While in no way admitting that the present claims are obvious over the claims of the cited applications, upon allowance of the claims of the instant application, Applicant will consider filing terminal disclaimers.

Claim Objections

Claims 2, 7, 16, and 25 stand objected to because the word "pregnenolone" is misspelled. Applicant, without acquiescence, has canceled claims 2 and 7 and has deleted the term "pregnanolone" throughout the remaining claims; thus, this basis for objection is moot and may be properly withdrawn.

Notice of Non-Compliant Amendment

The Action alleges that the amendment to the claims filed September 23, 2009, does not comply with the requirements of 37 C.F.R. § 1.121 (c) because the test of amended claim 22 filed on September 23, 2009, is not identified with the proper status identifier, but is identified as "currently amended withdrawn." Applicant respectfully submits that the present claim identifiers have been amended to comport with the requirements of 37 C.F.R. § 1.121 (c); thus, this basis of objection may be properly withdrawn.

In view of the preceding arguments, applicant believed that the present claims are in condition for allowance, which action is respectfully requested.

Should any additional fee be deemed due, please charge such fee to our Deposit Account No. 22-0261, reference our docket number 58086-241892, and notify the undersigned accordingly.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is hereby invited to telephone the undersigned at the number provided.

Respectfully submitted,

Date: **April 9, 2010**

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